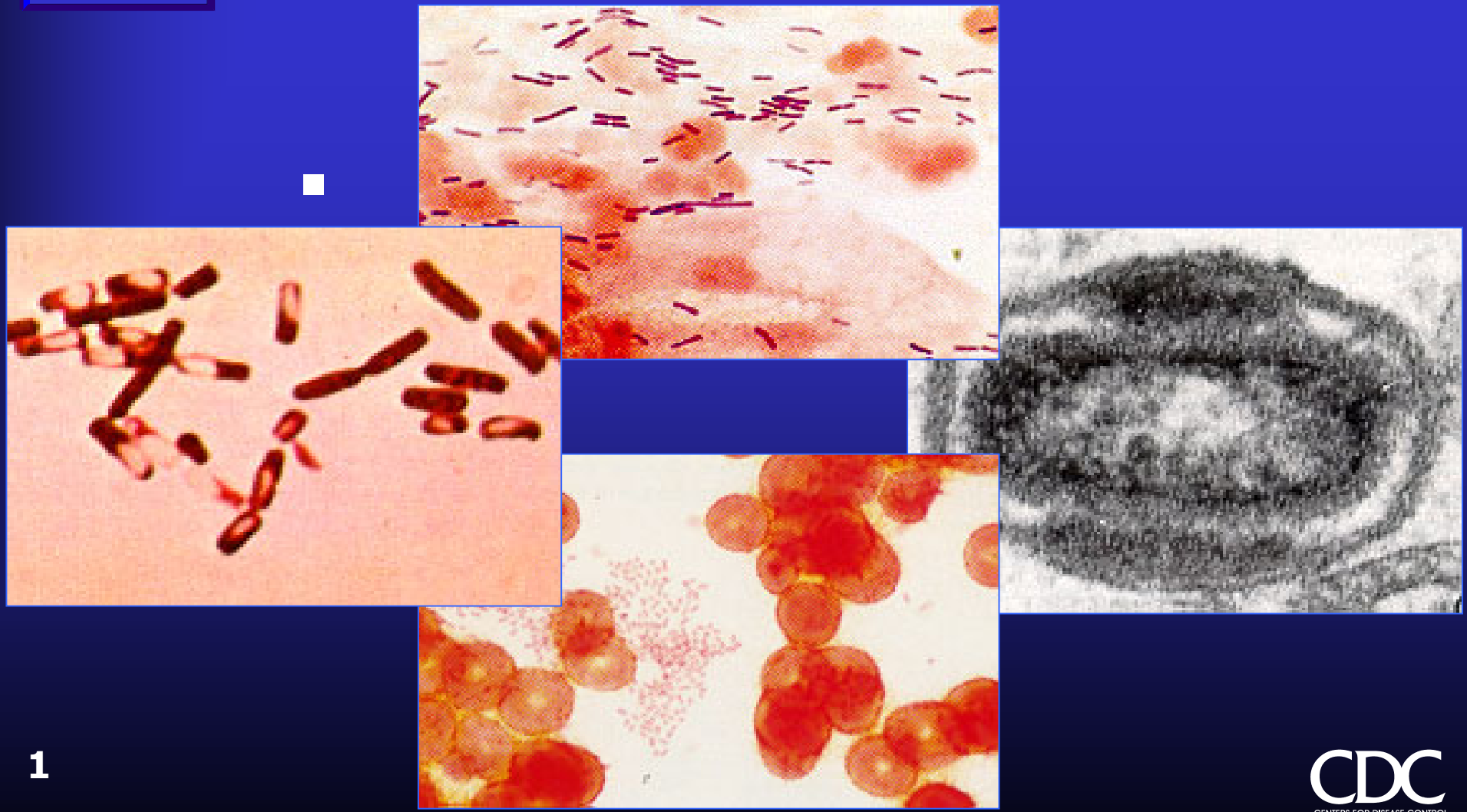
Agents of Bioterrorism



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Objectives

- Apply appropriate biosafety practices when working with potentially dangerous organisms
- Recognize agents that could be used for bioterrrorism and perform selected tests to rule them out

Objectives

- Refer potentially dangerous specimens or organisms to an appropriate lab for confirmation
- Develop an Emergency Preparedness Plan for your laboratory.

Level A Laboratory: Definition

- BSL-2 Laboratory with a certified Class II biological safety cabinet
  - BSL-1 microbiology practices plus
  - Directed by competent scientists
  - Personnel specifically trained in handling pathogenic agents

Level A Laboratory: Definition

- BSL-2 Laboratory with a certified Class II biological safety cabinet
  - Physical containment practices to minimize infectious aerosols
  - PPE

Role of the Level A Laboratory

- Rule out critical biological agents
- Refer to higher level laboratory

Bioterrorism Agents: Laboratory Risk

Agent	BSL	Laboratory Risk
B. anthracis	2	low
Y. pestis	2	medium
Brucella spp.	2/3	high
F. tularensis	2/3	high
Botulinum toxin	2	medium
Smallpox	4	high
Viral Hemorrhagic fever	4	high

Francisella tularensis

Tularemia

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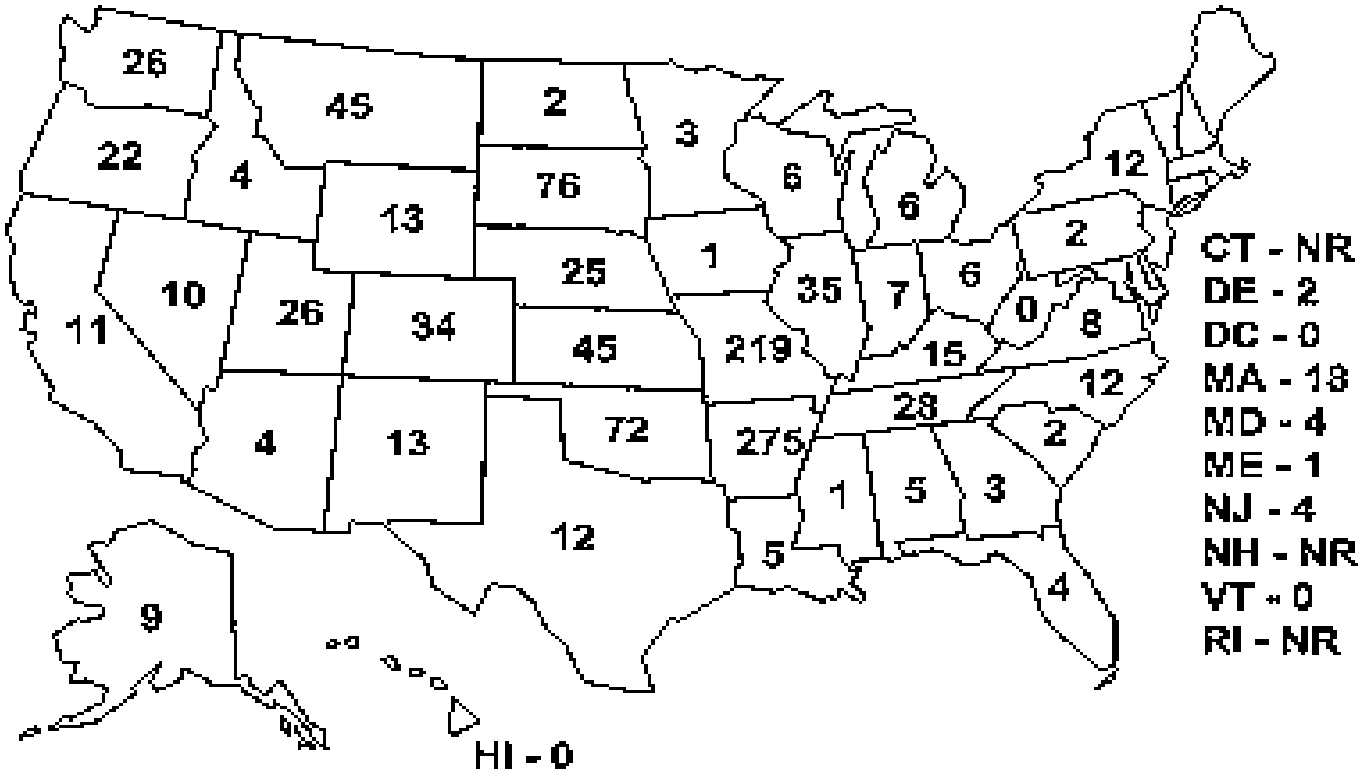
Francisella tularensis

"A Rose by Any Other Name"

- Plague-like disease in rodents (California)
- Deer-fly fever (Utah)
- Glandular tick fever (Idaho and Montana)
- Market men's disease (Washington, DC)
- Rabbit fever (Central States)
- O'Hara's disease (Japan)

Reported Cases of Tularemia - 1990-1998



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Tularemia

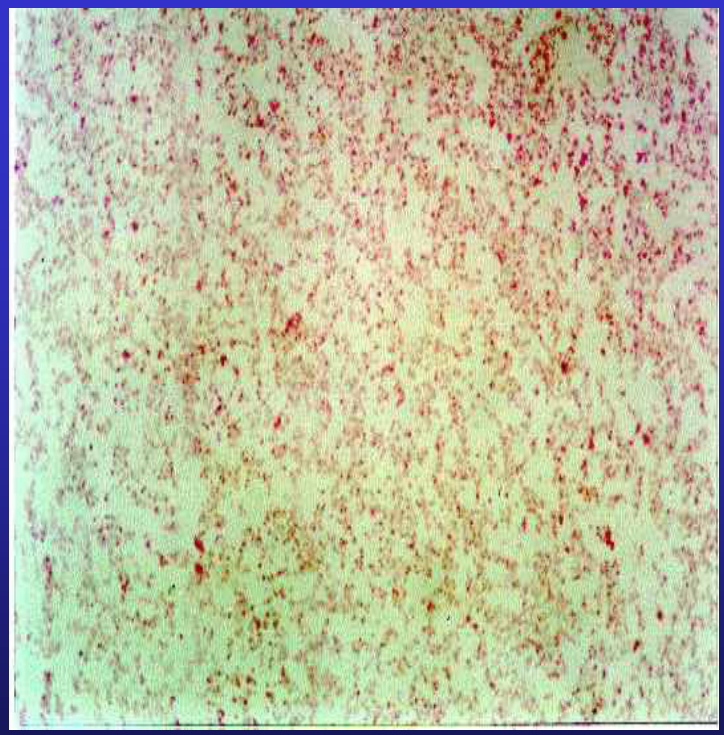
- Contagious --- no
- Infective dose --- 10-50 organisms
- Incubation period --- 1-21 days (average=3-5 days)
- Duration of illness --- ~2 weeks
- Mortality --- treated : low
  untreated: moderate
- Persistence of organism ---months in moist soil
- Vaccine efficacy --- good, ~80%

Level A Procedures
Francisella tularensis

- This is a dangerous, highly virulent organism and it should not be manipulated at the bench.
- Gram stain
- Growth characteristics in broth
- Growth characteristics in agar

Francisella tularensis

- Gram stain
  - Poorly staining, tiny Gram-negative coccobacilli



Francisella tularensis Growth Characteristics

- Fastidious, requires cysteine for robust growth: Cysteine Heart Agar (CHA) is ideal
  - Enriched chocolate agar : 9% sheep blood + cysteine
  - Not part of Level A routine procedures

Francisella tularensis Growth Characteristics

Gram Negative Coccobacilli

- **Most likely**
  - Acinetobacter
  - Actinobacillus
  - H. aphrophilus
  - Bordetella spp.
  - Pasturella spp.
- **Least likely**
  - DF-3
  - Brucella spp.
  - Francisella spp.

Francisella tularensis Technical Hints

If you see:

- Tiny, gram-negative coccobacilli from blood, lymph node aspirate, or respiratory specimens
- Blood isolates that grow slowly on chocolate agar but poorly on blood agar
- Robust growth in BCYE; requires cysteine

Refer

Yersinia pestis

Plague

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Plague Epidemiology

- U.S. averages 13 cases/yr (10 in 1998)
- 30% of cases are in Native Americans in the Southwest. 15% case fatality rate
- Most cases occur in summer

Plague Epidemiology

- Three Clinical Types:
  - bubonic (infected lymph nodes)
  - septicemic (blood-borne organisms)
  - pneumonic (transmissible by aerosol; deadliest)

Yersinia pestis Specimen Selection

- Specimen selection is important
  - Bubonic - bubo - lymph node aspirate
  - Septicemic - blood - Obtain three sets 10-30 minutes apart
  - Pneumonic
    - Sputum/throat
    - Bronchial washings

Yersinia pestis Specimen inoculation

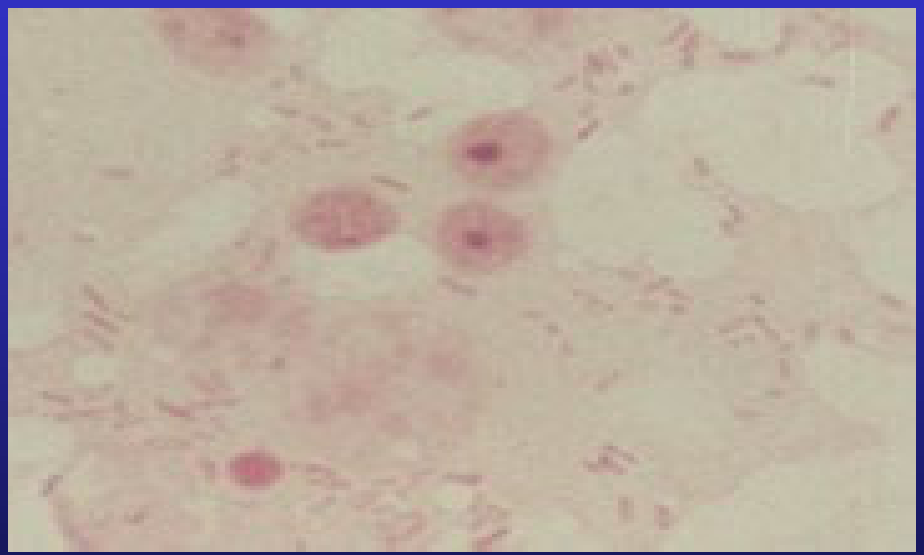
- Inoculate routine plating media and make thin smear for DFA
  - Use Wayson only if DFA is unavailable

Level A Procedures
Yersinia pestis

- Gram stain
- Wayson stain
- Growth characteristics on agar
- Growth characteristics in broth

Yersinia pestis Gram stain

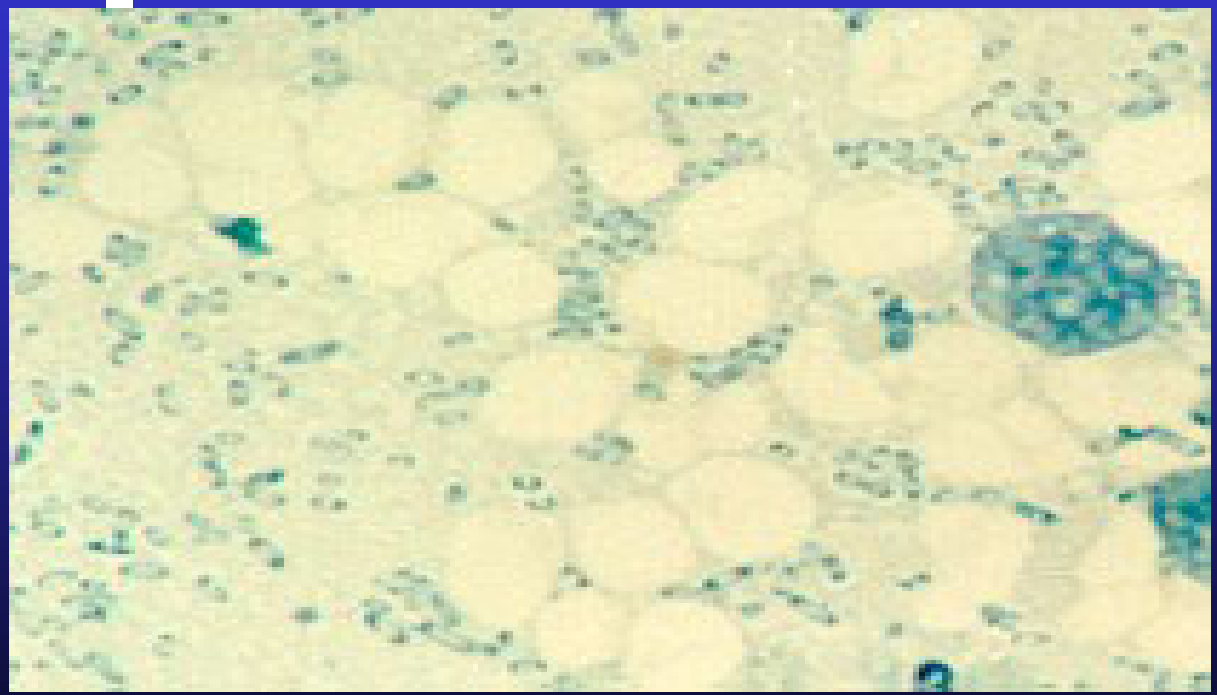
- Small, gram-negative bipolar-stained coccobacilli



Must confirm by DFA and mouse inoculation

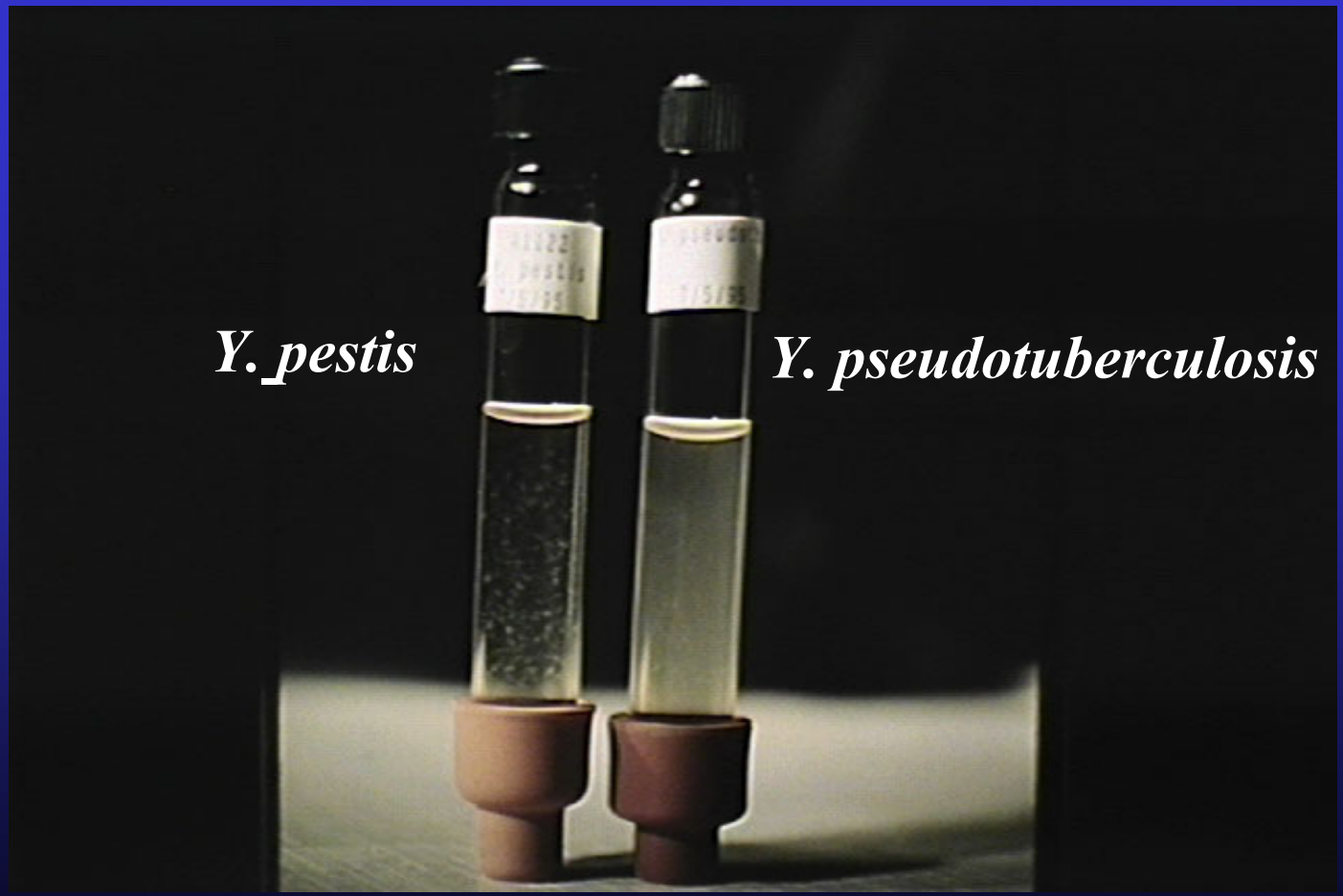
Yersinia pestis Wayson Stain

- Pink-blue cells with a closed safety pin look



Wayson stain alone is not diagnostic

Yersinia pestis in BHI Broth



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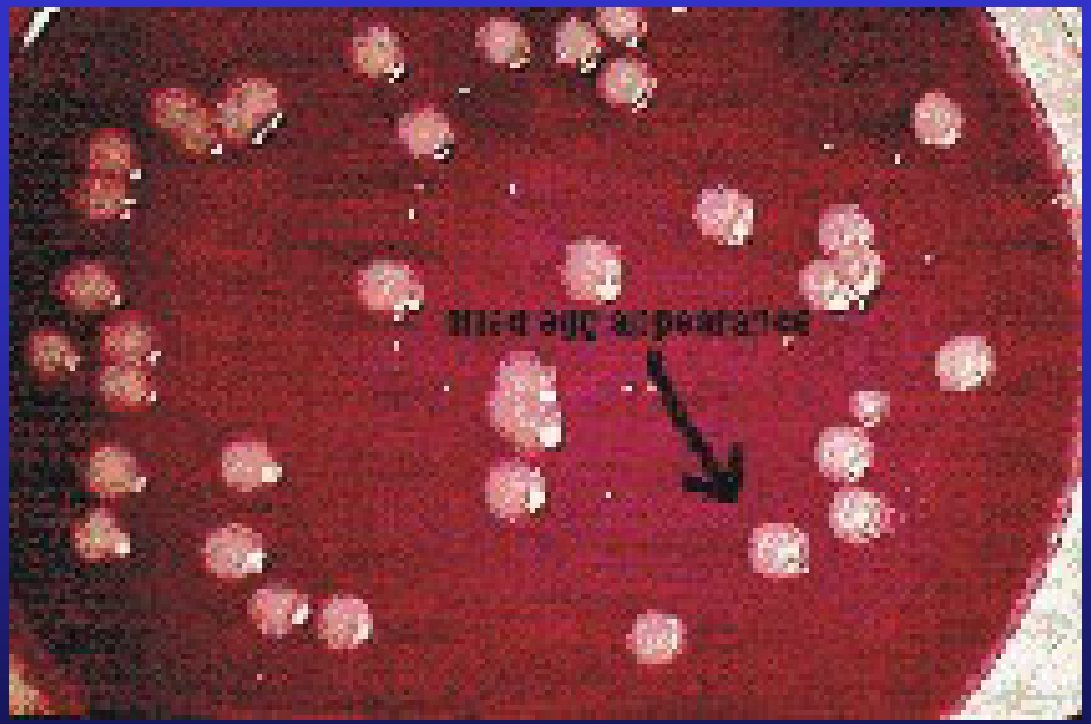
Yersinia pestis Technical Hints

- Small gram-negative, poorly staining rods from blood, lymph node aspirate, or respiratory specimens
- Safety pin appearance in Gram, Wright, Giemsa, or Wayson stain

Refer

Yersinia pestis blood plate



Variola virus

Smallpox

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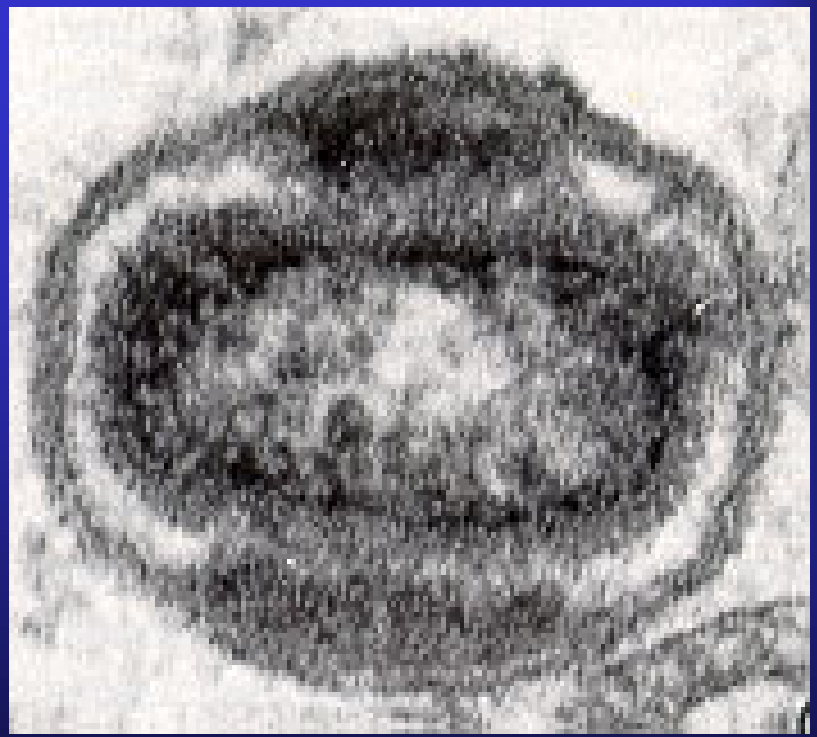
Variola
Smallpox virus

- Large DNA virus
- Dumbbell-shaped core
- Complex membranes



Refer

Level A Procedures Smallpox virus

- Rule out chickenpox (PCR)!
- Specimen of choice is lesion material from pustules
- Contact your State Public Health Laboratory for guidance

Hemorrhagic Fever Viruses

Ebola

Marburg

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Hemorrhagic Fever Viruses

- Families Responsible for VHF:
  - Arenaviridae
  - Bunyaviridae
  - Filoviridae
  - Flaviviridae

Hemorrhagic Fever Viruses

- Arenaviruses
  - Argentine Hemorrhagic Fever
  - Bolivian Hemorrhagic Fever
  - Sabia Associated Hemorrhagic Fever
  - Lassa Fever

Hemorrhagic Fever Viruses

- Bunyaviruses
  - Crimean-Congo Hemorrhagic Fever
  - Rift Valley Fever
  - Hantavirus Pulmonary Syndrome Hemorrhagic Fever

Hemorrhagic Fever Viruses

- Filoviruses
  - Ebola Hemorrhagic Fever
  - Marburg Hemorrhagic Fever

Hemorrhagic Fever Viruses

- Flaviviruses
  - Tick-borne Encephalitis
  - Kyasanur Forest Disease
  - Omsk Hemorrhagic Fever

Viral Hemorrhagic Fevers

- Contagious --- Moderate
- Infective dose --- 1-10 particles
- Incubation period --- 4-21 days
- Duration of illness --- 7-16 days
- Mortality ---variable
- Persistence of organism --- unstable
- Non-endemic in U.S.
- No vaccine

VHF Specimens

- Diagnosis is clinical, not laboratory
- No specimen accepted without prior consultation

Refer

Handling VHF Specimens

- Sample for serology - 10-12 ml
  - ship on dry ice
- Tissue for immunohistochemistry
  - formalin-fixed or paraffin block
  - ship at room temperature
- Tissue for PCR/virus isolation
  - ante-mortem, post-mortem; ship on dry ice
- ship serum cold or on dry ice in a plastic tube

Brucella spp.

Brucellosis

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BRUCELLOSIS

- Zoonotic disease caused by any of 4 Brucella spp.: *abortus*, *melitensis*, *suis*, and *canis*
- Systemic infection characterized by an undulant fever pattern
- Relatively rare in the U.S. with approximately 100 cases/year

Brucellosis
(by year, United States, 1966-1996)

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BRUCELLOSIS: HISTORY

- 1887 Bruce - Malta fever, M. melitensis
- 1897 Bang - cattle abortion, B. abortus
- 1914 Traum - sow, B. suis
- 1920 Evans, Meyer, Shaw - Brucella
- 1954 B. suis, first weaponized U.S. agent
- 1968 Carmichael - Beagles, *B. canis*

BRUCELLOSIS: TRANSMISSION

- Ingestion
  - The most common mode of transmission
- Direct skin contact/puncture
  - Occupational hazard for farmers, butchers and veterinarians
- Aerosols
  - Highly infectious

BRUCELLOSIS

- Infective dose = 10 -100 organisms
- Incubation period = 5 days - > 6 months
- Duration of illness = weeks to months
- Fever, profuse sweating, malaise, headache and muscle/back pain
- No person to person transmission
- Mortality: < 5%
- Stable organisms

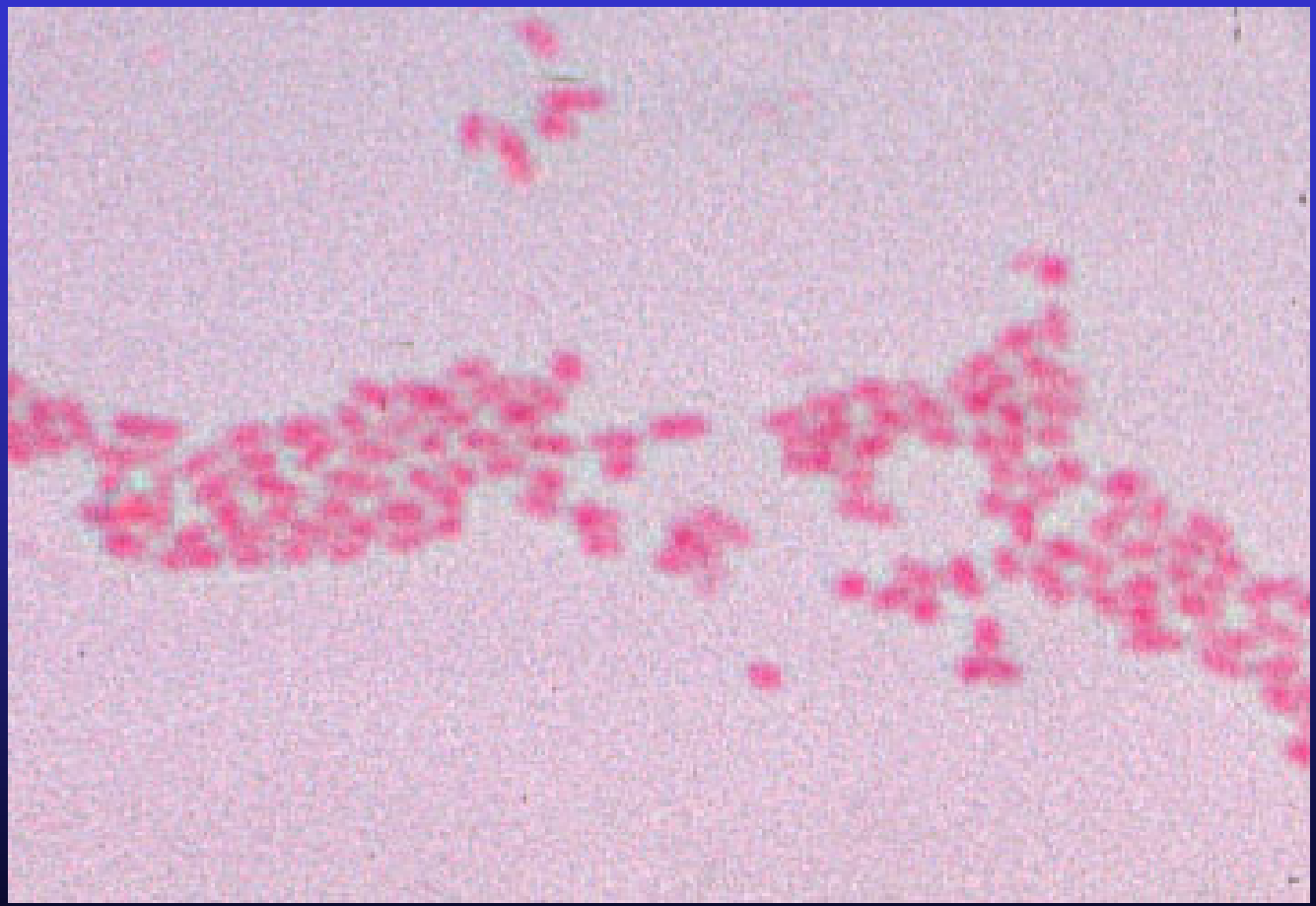
Brucella spp. Specimen Selection

- Serum
- Blood or bone marrow
- Tissue (spleen, liver)

Level A Laboratory Tests *Brucella* spp.

- Colonial morphology on SBA
- Gram stain morphology
- Oxidase
- Urea hydrolysis

B. abortus, gram stain (x3200)



Brucella spp. Key Level A Lab Tests

- Colonial morphology on SBA
  - Fastidious
  - Visible growth may take 48 - 72 hrs
  - Small (0.5-1.0mm), convex, glistening
  - Non-hemolytic and non-pigmented

Brucella spp. Key Level A Lab Tests

- Oxidase-positive
  - *B. melitensis* (100%)
  - *B. abortus* (96%)
  - *B. suis* (95%)
  - *B. canis* (72%)
- Urea hydrolysis-positive
  - *B. suis & B. canis* ~15 min
  - *B. abortus & B. melitensis* ~24hr

Brucella spp.
Other Ox+/Ur+ GN Oxidizers

- Achromobacter grp B
- Acidovorax spp
- Agrobacterium spp
- EO-2/EO-3
- Flavobacterium spp
- Methylobacterium spp
- Ochrobactrum
- Pseudomonas spp
- Riemerella
- Roseomonas spp
- O-2
- II-i

Brucella spp. Technical Hints

- **Misidentified as *Moraxella sp.***
  - **Clin Inf Dis 1993; 17:1068-9**
- Reported as gram-positive cocci, mistaken for “slow-growing” *Staphylococcus sp.*

Brucella spp. Review of Key Tests

- Tiny, faintly staining, gram-negative coccobacilli from blood or bone marrow
- Oxidase +
- Urease +

REFER

Clostridium botulinum

Botulism

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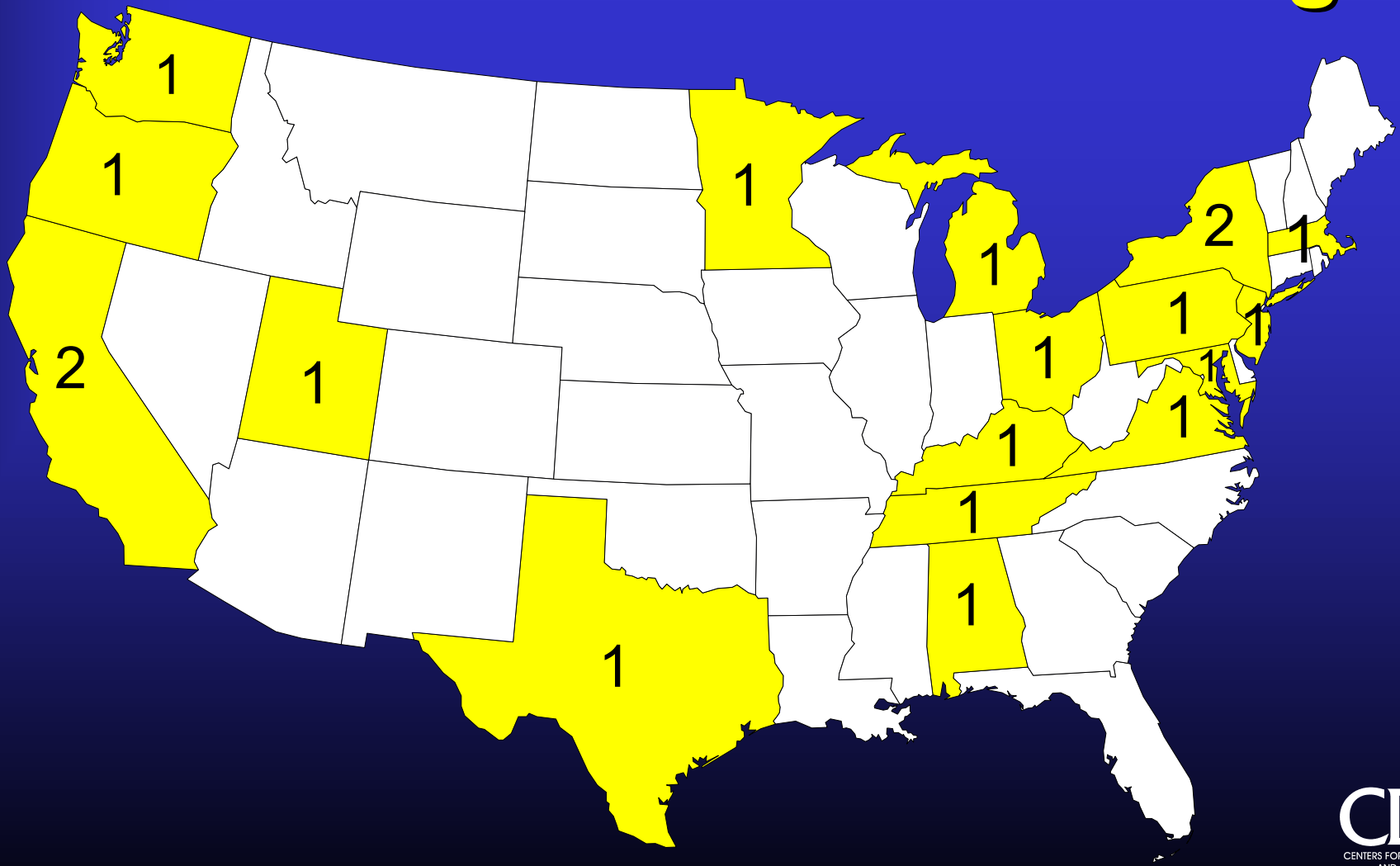
BOTULISM

- Diagnosis of botulism is made clinically
- Health care providers suspecting botulism should contact their State Health Department

REFER

Laboratory Capacity for Botulinum Toxin Testing



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FOODBORNE BOTULISM

- Infective dose: 0.001 μg/kg
- Incubation period: 18 - 36 hours
- Dry mouth, double vision, droopy eyelids, dilated pupils
- Progressive descending bilateral muscle weakness & paralysis
- Respiratory failure and death
- Mortality 5-10%, up to 25%

FOODBORNE BOTULISM

- Among 309 persons with clinically diagnosed botulism reported to CDC from 1975 to 1988:
  - Stool cultures for C. botulinum: 51% +
  - Serum botulinum toxin testing: 37% +
  - Stool botulinum toxin testing: 23% +
- Overall, at least one of the above tests was positive for 65% of all patients

Level A Procedures for Botulism Event

- Properly collected specimens are to be referred to designated testing laboratories
- Prior to the shipment of any botulism-associated specimen, the designated laboratory must be notified and approved by the State Health Department

Level A Procedures for Botulism Event

- Clinical specimens to be collected:
  1. Serum
  2. Gastric contents or vomitus
  3. Feces or return from sterile water enema
  4. Wound tissue

Botulism Biosafety Alert

- Botulism toxins are extremely poisonous
- Minute quantities acquired by ingestion, inhalation, or by absorption can cause death
- All materials suspected of containing toxin must be handled with CAUTION!

REFER

Bacillus anthracis

Anthrax

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ANTHRAX

- Three forms of human anthrax occur:
  1. Cutaneous
  2. Gastrointestinal
  3. Inhalational

Cutaneous anthrax

Vesicle development, day 2

Eschar formation, day 4

www.who.int/emc/

Anthrax Lesion on Neck

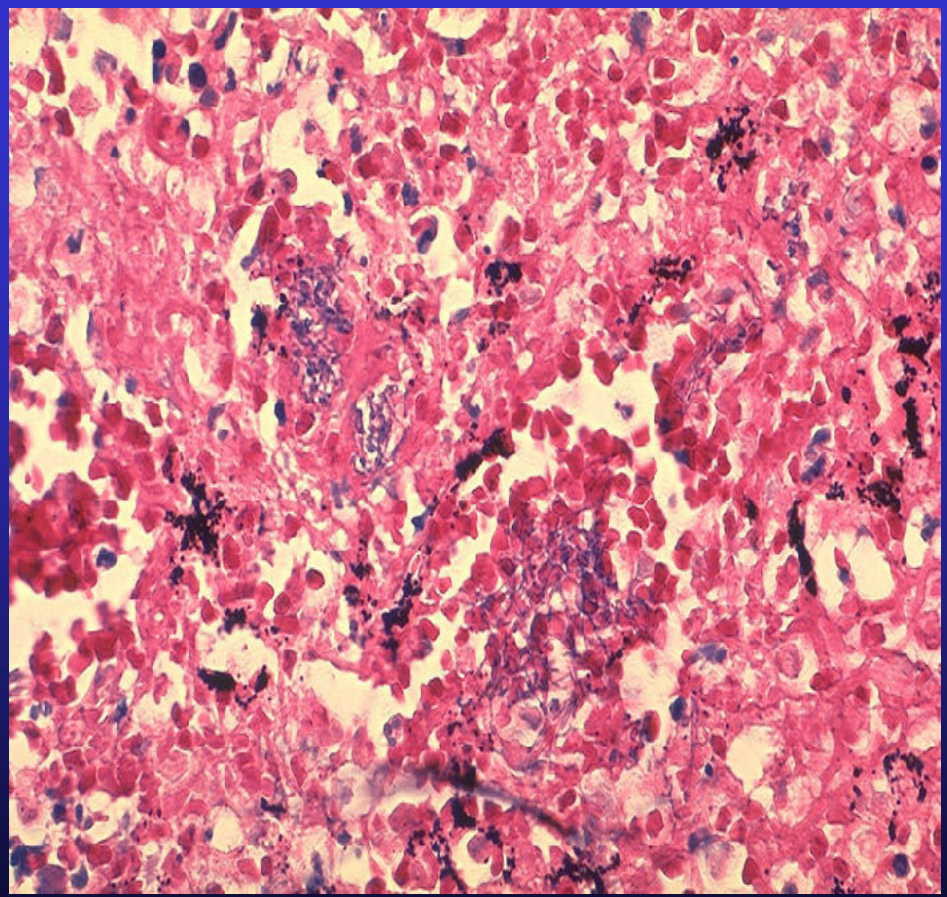
Inhalational Anthrax

- Infective dose = 8,000 - 15,000 spores
- Incubation period = 1-6 days
- Duration of illness = 3-5 days
- Fever, malaise, and fatigue
- Short period of improvement = up up to 2 days
- Abrupt respiratory distress…death <24hrs
- No person to person transmission

Inhalational Anthrax

- Infection of tissue
- Late in the course of infection
- Chest X-ray

Mediastinal LN, microcolonies of *B anthracis*, Giemsa stain



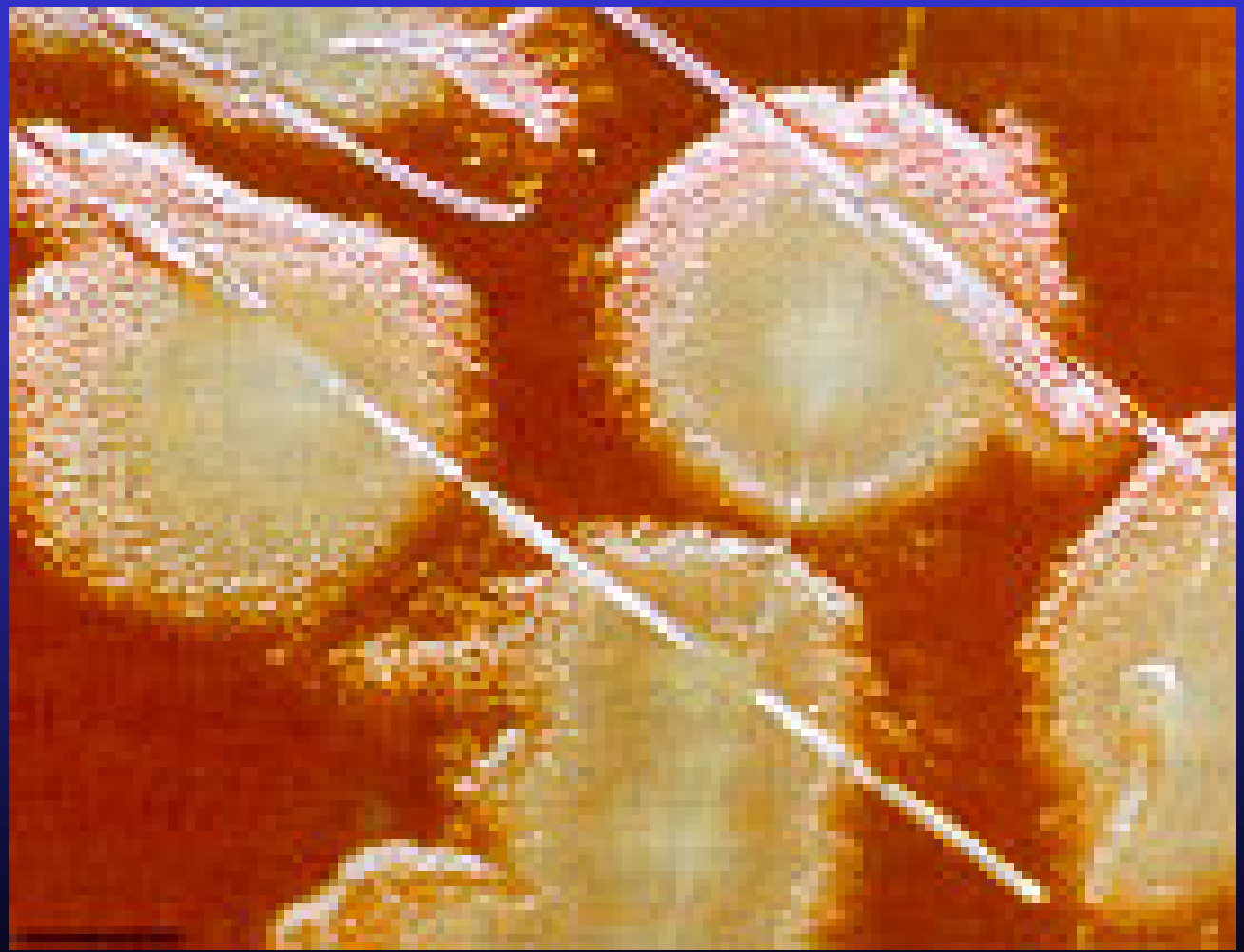
Anthrax: Specimen Selection

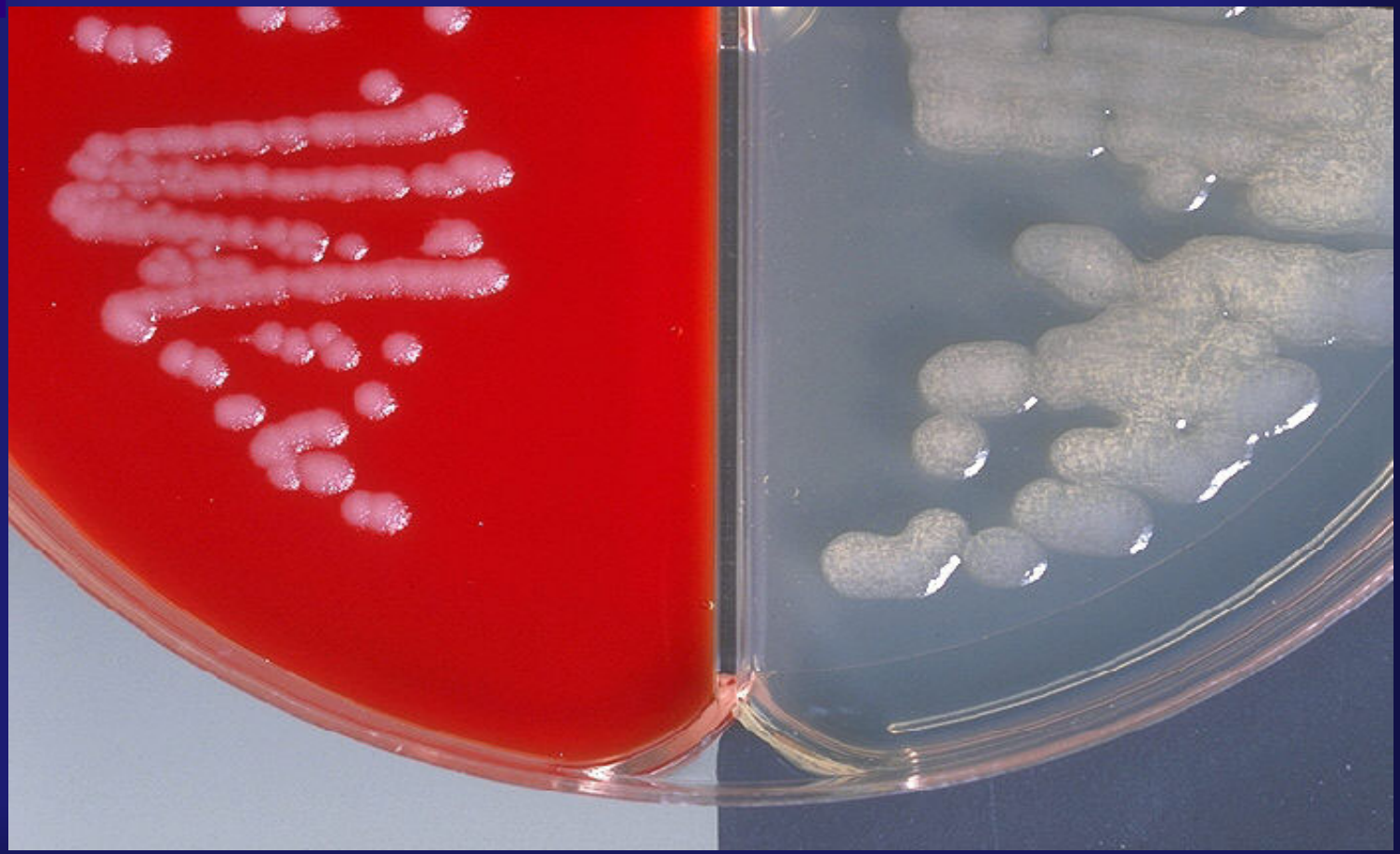
- Inhalation: Sputum and Blood
- Cutaneous: Vesicles and Eschar
- Gastrointestinal: Stool and Blood

Bacillus anthracis Key Level A Lab Tests

- Gram stain
- Growth characteristics on agar
- Sporulation, in air
- Lack of motility
- Penicillin inhibition zone
- Capsule formation

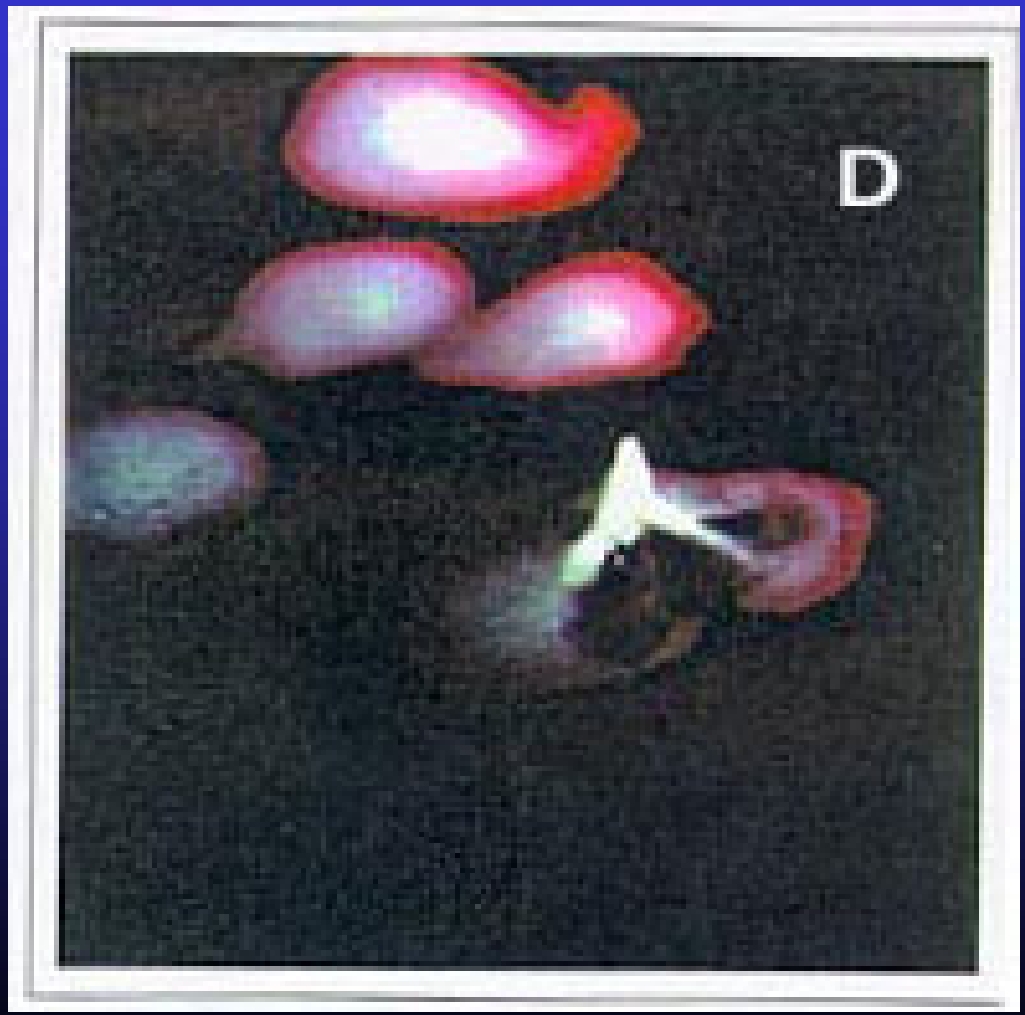
B. anthracis Colony on SBA





B. anthracis on blood (left) and bicarbonate agar (right) plate (demonstrating capsule formation)

"STICKY" consistency of *B. anthracis'* colony on SBA



B. anthracis, Gram stain demonstrating spores

Bacillus anthracis Presumptive Identification

- Gram-positive rod
- catalase-positive
- Aerobic spore formation

Bacillus anthracis Presumptive Identification

- Spores are oval
- non-swelling of vegetative cell
- ground glass colony morphology
  - *B. anthracis (non-motile)*
  - *B. cereus*
  - *B cereus var mycoides (non-motile)*
  - *B. thuringiensis*

Bacillus anthracis Presumptive Identification

- Nonmotile: *B anthracis* and *B cereus* var mycoides (and *B. megaterium*)
- Nonhemolytic, penicillin inhibition zone (15-20 mm)
- Capsule formation

REFER

CDC Laboratory Pearl

- The most common *Bacillus sp.* submitted to CDC to r/o *B. anthracis* are non-motile *B. megaterium*

- A non-motile, *Bacillus sp,* is recovered from a blood culture. What is one of the fastest procedures to r/o anthrax ?

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